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Platinum(II) complexes with R_2edda ligands (R = Me, Et, *n*-Pr; edda = ethylenediamine-*N*,*N*′-diacetate): Synthesis and characterization

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Dedicated to Professor Vukadin Leovac on the occasion of his 70th birthday.

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ABSTRACT

Three novel complexes of platinum(II) with R_2edda bidentate ligands [$PtCl_2(R_2edda)$] (R = Me, Et, *n*-Pr; edda = ethylenediamine-*N*,*N*′-diacetate; **1**–**3**), are synthesized and characterized by IR and NMR spectroscopy and elemental analysis. All complexes exist in three stereoisomeric forms (*R*,*R*), (*S*,*S*) and (*R*,*S*)≡(*S*,*R*). In addition crystal structure of one platinum(IV) complex [$PtCl_4((n\text{-}Pr)_2edda)$], **4**, is presented. Furthermore, in order to assign stereoisomers of **1**–**3**, a reduction of racemic [$PtCl_4(Et_2edda)$] by ascorbic acid to [$PtCl_2(Et_2edda)$] (**2**) was performed and analyzed by ^{1}H NMR. Time-depending ^{1}H NMR spectroscopic experiments were implemented to study the stability of ethylenediamine-*N*,*N*′-diacetate diesters. Finally, the *in vitro* cytotoxic activity of complexes **1**–**3** was studied on 11 tumor cell lines, 518A2 (melanoma), 8505C (human thyroid carcinoma), A253 (head and neck tumor), A431 (cervix), A549 (lung), A2780 (ovarian), MCF-7 (breast) and HT-29, HCT-8, DLD-1, SW1736 (all colon) by the SRB colorimetric assay method. Complex **3** showed the highest action against ovarian (A2780) cells with an IC_{50} value 51 ± 1 μM.

1. Introduction

As cisplatin still remains the most successful anticancer drug [1,2], the last 4 decades are marked by a growing interest for synthesis of platinum complexes with bidentate nitrogen ligands. Platinum(II) and platinum(IV) complexes with *O*,*O*′-dialkyl-ethylenediamine-*N*,*N*′-diacetate, R_2edda, and *O*,*O*′-dialkyl-ethylenediamine-*N*,*N*′-di-3-propanoate (R_2eddp; and their analogs) ligands, abbreviated as R_2edda-type (Fig. 1), are interesting not only in the aspect of synthesis and characterization of new compounds, but also as potential antitumor drugs and some significant results are gathered and published [3–7].

Recently, preparation and antitumor action of two analogues of these high active platinum(IV) complexes with shorter chain in ester function, [$PtCl_4(R_2eddp)$] (R = Et and *n*-Pr), was reported. Studies revealed that platinum(IV) complexes may act as drugs by interacting with DNA, or prodrugs that can be activated *in vivo* in the cell, mostly by reduction of platinum(IV) to corresponding reactive platinum(II) compounds [5].

Structurally, these complexes are very intriguing as nitrogen atoms become stereocenters by coordinating, thus, investigation of feasible diastereoisomers is possible. Their stereochemistry is investigated in traditional manner by X-ray structural analysis and NMR spectroscopy and supported by quantum chemical calculations. Whereas, in case of platinum(IV) complexes presented in Fig. 1. **A** and **B**, only enantiomeric pairs (*R*,*R*)-*N*,*N*′, (*S*,*S*)-*N*,*N*′ were observed, for **C** and **D** only (*R*,*R*)-*N*,*N*′-configuration isomers were formed. On the other hand for platinum(II) complexes all three stereoisomers are obtained as shown in Fig. 2 [4–7].

R_2edda-type esters are a family of *O*,*O*′-dialkyl esters of ethylenediamine-*N*,*N*′-diacetic/dipropanoic/dibutanoic/dipentanoic acids and coordinate in κ^2N,N' manner to platinum forming five-membered chelates. Therefore, complexes are easily obtained in reaction of single platinum salts and desired ligand precursors, in the presence of lithium hydroxide. There has been evidence of hydrolysis of ester groups and coordination in $\kappa O,\kappa^2N,N'$ or κ^2-*O*,*O*′,κ^2N,N' mode to metal ions, thus hydrolytic stability of esters is questionable [4,8].

Herein we describe synthesis and characterization of three novel platinum(II) complexes with R_2edda ligands [$PtCl_2(Me_2edda)$], **1**, [$PtCl_2(Et_2edda)$], **2**, and [$PtCl_2((n\text{-}Pr)_2edda)$], **3**. The crystal

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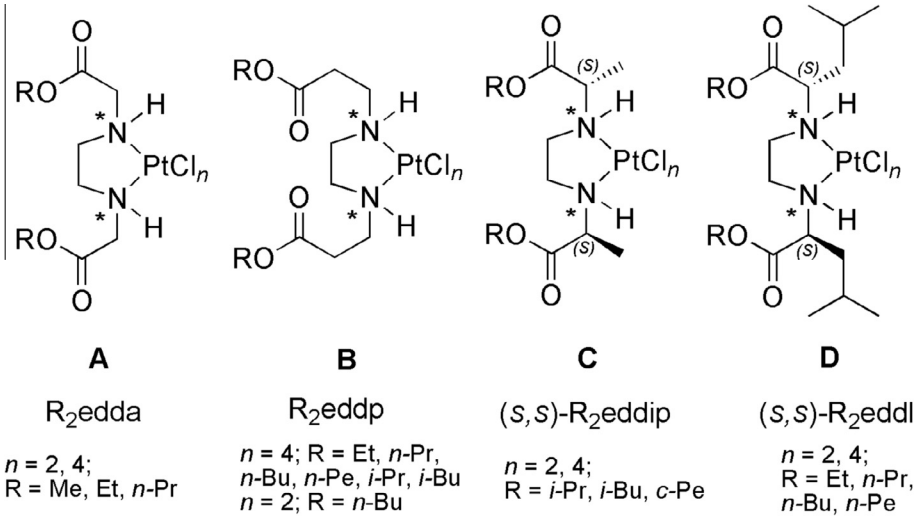


Fig. 1. Complexes of platinum(IV) and platinum(II) with R_2edda-type ligands. (*S*,*S*)-R_2eddip = (*S*,*S*)-*O*,*O*′-dialkyl-ethylenediamine-*N*,*N*′-di-2-propanoate; (*S*,*S*)-R_2eddl = (*S*,*S*)-*O*,*O*′-dialkyl-ethylenediamine-*N*,*N*′-di-2-(4-methyl)pentaoate (**N* stereocenter).

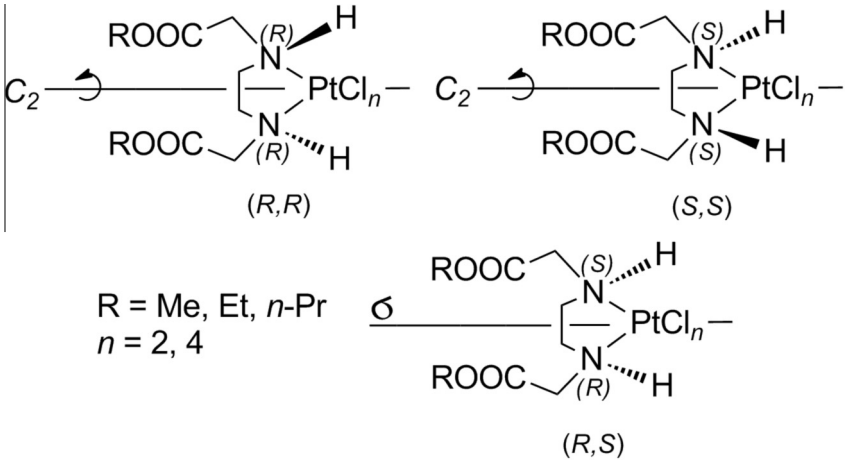


Fig. 2. Possible isomers of [$PtCl_n(R_2edda)$] (n = 2 or 4) and information on their symmetry.

structure of one platinum(IV) complex, [$PtCl_4$((*n*-Pr)$_2$edda)], **4** is reported. Furthermore, reduction of *rac*-[$PtCl_4$(Et_2edda)] by ascorbic acid to [$PtCl_2$(Et_2edda)] (**2**) was monitored by NMR spectroscopy, in order to assign diasteroisomers of platinum(II) complexes **1**–**3**. Reactivity of ethylenediamine-*N*,*N*′-diacetate diesters, in terms of hydrolysis and cyclization is studied by ^{1}H NMR spectroscopic experiments. *In vitro* cytotoxic activity was determined for **1**–**3** against 11 tumor cell lines: 5182A (melanoma), 8505C (human thyroid carcinoma), A253 (head and neck tumor), A431 (cervix), A549 (lung), A2780 (ovarian), MCF-7 (breast) and HT-29, HCT-8, DLD-1, SW1736 (all colon) by the SRB colorimetric assay method.

2. Experimental

2.1. Materials and methods

The elemental analyses (C, H, N) were carried out on a CHNS-93 (LECO) elemental analyzer. Infrared spectra were recorded in KBr pellets on a Mattson Galaxy 5000 FT-IR spectrometer, covering the region 4000–300 cm^{-1}. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 400 NMR spectrometer in DMF-d_7, acetone-d_6, methanol-d_4 or D_2O at 27 °C. Dimethyl-, diethyl- and di-*n*-propyl-ethylenediamine-*N*,*N*′-diacetate dihydrochloride, R_2edda·2HCl; R = Me (**L1**·2HCl), Et (**L2**·2HCl), *n*-Pr (**L3**·2HCl), were synthesized by procedures reported in literature [4,9].

2.2. General procedure for the preparation of [$PtCl_2(R_2edda)$] complexes *1*–*3*

K_2[$PtCl_4$] (0.091 g, 0.22 mmol) was dissolved in 10 mL of water and the appropriate ligand dihydrochloride (**L1**·2HCl: 0.061 g; **L2**·2HCl: 0.067 g; **L3**·2HCl: 0.073, each 0.22 mmol) was added. The mixture was stirred for 5 h and during this period 3.92 mL LiOH (c = 0.112 mM, 0.44 mmol) were introduced. The complexes are obtained as yellow precipitates which were filtered off, washed with water (2 × 5 mL) and dried on air. Mixture of isomers were obtained and denoted as (**1**–**3**) **a** [(*R*,*R*)/(*S*,*S*)] and (**1**–**3**)**b** [(*R*,*S*)≡(*S*,*R*)].

1: Yield 0.069 g, 67%. *Anal.* Calc. for $C_8H_{16}Cl_2N_2O_4Pt$ (M_r 470.21): C, 20.43; H, 3.43; N, 5.96. Found: C, 20.23; H, 3.72; N, 5.78%. **1a**: ^{1}H NMR (500 MHz, DMF-d_7): δ = 3.10 (m, 4H, CH_2), 3.59 (s, 6H, CH_3), 3.96 (m, 2H, CH_2-(en)), 4.24 (m, 2H, CH_2-(en)), 6.55 (br s, 2H, N*H*) ppm. ^{13}C NMR (DMF-d_7): δ = 51.8 (CH_2-(en)), 52.7 (CH_2), 54.5 (CH_3), 169.0 (*C*OO) ppm. **1b**: ^{1}H NMR (500 MHz, DMF-d_7): δ = 3.10 (m, 4H, CH_2), 3.62 (m, 2H, CH_2-(en)), 3.73 (s, 6H, CH_3), 4.28 (m, 2H, CH_2-(en)), 6.55 (br s, 2H, N*H*) ppm. ^{13}C NMR (DMF-d_7): δ = 51.8 (CH_2-(en)), 52.7 (CH_2), 54.5 (CH_3), 169.0 (*C*OO) ppm.

Ratio of isomers **1a**/**1b** = 2/3. IR (KBr): ν 3147 (m), 2984 (m), 1734 (s), 1462 (m), 1220 (s), 1107 (s), 821 (w), 426 (m) cm^{-1}.

2: Yield 0.068 g, 62%. *Anal.* Calc. for $C_{10}H_{20}Cl_2N_2O_4Pt{\cdot}H_2O$ (M_r 516.28): C, 23.26; H, 4.30; N, 5.43. Found: C, 23.57; H, 4.25; N, 5.46%. **2a**: ^{1}H NMR (500 MHz, DMF-d_7): δ = 1.23 (s, 6H, CH_3), 2.79 (m, 4H, CH_2), 3.71 (m, 2H, CH_2-(en)), 4.18 (m, 4H, CH_2O), 4.18 (m, 2H, CH_2-(en) (superimposed with CH_2O signal)), 6.46 (br s, 2H, N*H*) ppm. ^{13}C NMR (DMF-d_7): δ = 14.2 (CH_3), 53.4 (CH_2-(en)), 55.0 (CH_2), 61.6 (CH_2O), 169.1 (COO) ppm. **2b**: ^{1}H NMR (500 MHz, DMF-d_7): δ = 1.23 (s, 6H, CH_3), 3.16 (m, 4H, CH_2), 3.92 (m, 2H, CH_2-(en)), 4.18 (m, 4H, CH_2O), 4.33 (m, 2H, CH_2-(en)), 6.46 (br s, 2H, N*H*) ppm. ^{13}C NMR (DMF-d_7): δ = 14.2 (CH_3), 54.1 (CH_2-(en)), 55.9 (CH_2), 61.6 (CH_2O), 169.2 (COO) ppm. Ratio of isomers **2a**/**2b** = 1/2. IR (KBr): ν 3142 (m), 2969 (m), 1741 (s), 1458 (m), 1218 (s), 1103 (s), 820 (w), 423 (m) cm^{-1}.

3: Yield 0.070 g, 60%. *Anal.* Calc. for $C_{12}H_{24}Cl_2N_2O_4Pt$ (M_r 526.32): C, 27.38; H, 4.60; N, 5.32. Found: C, 27.25; H, 4.85; N, 5.22%. **3a**: ^{1}H NMR (500 MHz, DMF-d_7): δ = 0.89 (t, 6H, CH_3), 1.62 (m, 4H, CH_2-Pr), 2.79 (m, 4H, CH_2), 3.73 (m, 2H, CH_2-(en)), 4.07 (m, 4H, CH_2O), 4.19 (m, 2H, CH_2-(en)), 6.45 (br s, 2H, N*H*) ppm. ^{13}C NMR (DMF-d_7): δ = 10.4 (CH_3), 22.3 (CH_2-Pr), 53.4 (CH_2-(en)), 55.0 (CH_2), 67.2 (CH_2O), 169.5 (COO) ppm. **3b**: ^{1}H NMR (500 MHz, DMF-d_7): δ = 0.89 (t, 6H, CH_3), 1.62 (m, 4H, CH_2-Pr), 3.15 (m, 2H, CH_2), 3.94 (m, 2H, CH_2-(en)), 4.07 (m, 4H, CH_2O), 4.32 (m, 2H, CH_2-(en)), 6.45 (br s, 2H, N*H*) ppm. ^{13}C NMR (DMF-d_7): δ = 10.4 (CH_3), 22.3 (CH_2-Pr), 54.1 (CH_2-(en)), 55.9 (CH_2), 67.2 (CH_2O), 169.6 (COO) ppm. Ratio of isomers **3a**/**3b** = 2/3. IR (KBr): ν 3120 (m), 2976 (m), 1742 (s), 1453 (m), 1224 (s), 1109 (s), 828 (w), 424 (m) cm^{-1}.

2.3. X-ray structure determinations

Crystals suitable for X-ray analyses were grown from the reaction mixture described in [4] at room temperature over several days. Intensity data were collected on a STOE IPDS diffractometer at 220(2) K using graphite monochromatized Mo Kα radiation (λ = 0.71073 Å). A summary of the crystallographic data, the data collection parameters, and the refinement parameters is given in Table 1. Absorption correction was applied numerically (T_{min}/T_{max}: 0.37/0.64). The structure was solved by direct methods with SHELXS-96, refined using full-matrix least-squares routines against F^2 with SHELXS-97 and analyzed with PLATON [10–12]. Non-hydrogen atoms were refined with anisotropic displacement parameters. Two hydrogen atoms were found in the electron density map and the rest was placed in calculated positions with fixed displacement parameters (riding model) and all of them were refined isotropically. ORTEP-3 program has been used for representation of the structure [13].

Table 1
Crystallographic data for **4**.

	[$PtCl_4$((n-Pr)$_2$edda)]
Empirical formula	$C_{12}H_{24}Cl_4N_2O_4Pt$
M_r	597.22
Crystal system	monoclinic
Space group	$C2/c$
a (Å)	20.857(8)
b (Å)	7.869(2)
c (Å)	12.025(4)
α (°)	90
β (°)	97.35(4)
γ (°)	90
V (Å^3)	1957.5(11)
Z	4
D_{calc} (g cm^{-3})	2.026
μ (Mo Kα) (mm^{-1})	7.731
F(000)	1152
θ Range (°)	2.75–25.85
Reflections collected	6049
Reflections observed [$I > 2\sigma(I)$]	1332
Reflections independent	1699
Data/restraints/parameters	1699/17/123
Goodness-of-fit (GOF) on F^2	0.969
$R1$, $wR2$ [$I > 2\sigma(I)$]	R_1 = 0.0410, wR_2 = 0.0851
$R1$, $wR2$ (all data)	R_1 = 0.0588, wR_2 = 0.0797
Largest difference peak and hole/e Å^{-3}	1.798/−1.402

2.4. Reduction of rac-[$PtCl_4$(Et_2edda)] complex with ascorbic acid

In an NMR tube containing 15 mg of [$PtCl_4$(Et_2edda)] (0.025 mmol), synthesized by the literature procedure [4], in 0.7 mL deuterated acetone 50 mg (0.280 mmol; excess) of ascorbic acid was added. ^{1}H NMR spectra were recorded before and after addition (immediately, 1 and 2 days) of ascorbic acid. After 2 days reaction was completed yielding corresponding enantiomeric pair (*R*,*R*)/(*S*,*S*) (isomer **2a**) of platinum(II) complex **2**.

Yield: quantitative. ^{1}H NMR (500 MHz, acetone-d_6): δ = 1.23 (broad, 6H, CH_3), 3.48 (m, 4H, CH_2), 3.68 and 4.15 (m, 4H, CH_2-(en)), 4.15 (m, 4H, CH_2O).

2.5. ^{1}H NMR spectroscopic experiments to study the reactivity of ethylenediamine-*N*,*N*′-diacetate diesters

2.5.1. Dimethyl-ethylenediamine-*N*,*N*′-diacetate

L1·2HCl·H_2O (16 mg, 54 μmol) is suspended in methanol-d_4 (0.75 mL) at −78 °C. *n*-Butyllithium (68 μL, 108 μmol) in *n*-hexan (1.6 M) is added to methanol-d_4 (0.75 mL) at −78 °C and the formed lithium methoxide solution is added to the suspension of **L1**·2HCl·H_2O. The product in methanol-d_4 is stored at −78 °C and only warmed up during the NMR spectroscopic measurements.

Yield: quantitative. ^{1}H NMR (400 MHz, CD_3OD): δ = 2.80 (s, 4H, CH_2-(en)) 3.49 (s, 4H, CH_2), 3.74 (s, 6H, CH_3) ppm.

The NMR tube from the reaction described above with dimethyl-ethylenediamine-*N*,*N*′-diacetate was warmed up to room temperature. The reaction was monitored with ^{1}H NMR spectroscopy and after 2.5 h the reaction was half-finished. After 24 h only signals of the cyclic product (for assignment see Fig. 6) were observed.

Yield: quantitative. ^{1}H NMR (400 MHz, CD_3OD): δ = 3.17 (t, 2H, H^a), 3.50 (t, 2H, $H^{a'}$), 3.54 (s, 2H, H^b), 3.74 (s, 3H, H^c), 4.17 (s, 2H, $H^{b'}$) ppm.

2.5.2. Di-*n*-propyl-ethylenediamine-*N*,*N*′-diacetate dihydrochloride monohydrate

L3·2HCl·H_2O was dissolved in deuterium oxide and studied with ^{1}H NMR spectroscopy (within 2 months). Slow hydrolysis took place and the partially hydrolysed product, *n*-propyl-ethylenediamine-*N*-acetate-*N*′-acetic acid was observed.

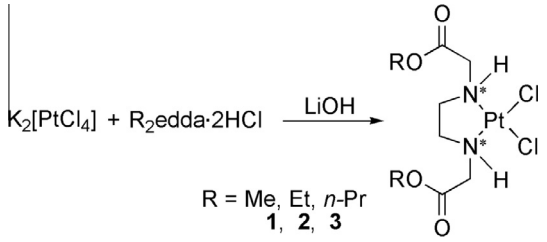
Yield: 50%. ^{1}H NMR (400 MHz, D_2O): δ = 0.97 (t, 3H, CH_3), 1.73 (q, 2H, CH_2CH_3), 3.67 (m, 2H CH_2-(en)), 3.82 (m, 2H, CH_2-(en)), 4.04 (s, 2H, CH_2), 4.21 (t, 2H, CH_2O), 4.32 (s, 2H, CH_2) ppm.

2.6. In vitro studies

The compounds **1**–**3** were dissolved in *N*,*N*-dimethyl formamide (DMF, Sigma Aldrich) to a concentration of 20 mM and diluted by nutrient medium to various working concentrations. Nutrient medium was RPMI-1640 (PAA Laboratories) supplemented with 10% fetal bovine serum (BiochromAG) and penicillin/streptomycin (PAA Laboratories).

2.6.1. Cell lines and culture conditions

The cell lines 518A2, 8505C, A253, A431, A549, A2780, MCF-7, HT-29, HCT-8, DLD-1 and SW1736 were included in this study. Cultures were maintained as monolayers in RPMI 1640 (PAA Laboratories, Pasching, Germany) supplemented with 10% heat inacti-



Scheme 1. Synthesis of platinum(II) complexes **1**–**3**.

vated fetal bovine serum (Biochrom AG, Berlin, Germany) and penicillin/streptomycin (PAA Laboratories) at 37 °C in a humidified atmosphere with 5% CO_2.

2.6.2. Cytotoxicity assay

The cytotoxic activities of the platinum compounds were evaluated using the sulforhodamine-B (SRB, Sigma Aldrich) microculture colorimetric assay [14]. In short, exponentially growing cells were seeded into 96 well plates on day 0 at the appropriate cell densities to prevent confluence of the cells during the period of experiment. After 24 h, the cells were treated with serial dilutions of the platinum compounds (0–125 μM) for 96 h. The final concentration of DMF solvent never exceeded 0.5%, which was non-toxic to the cells. The percentages of surviving cells relative to untreated controls were determined 96 h after the beginning of drug exposure. After 96 h treatment, the supernatant medium from the 96 well plates was thrown away and the cells were fixed with 10% TCA. For a thorough fixation plates are now allowed to stand at 4 °C. After fixation the cells are washed in a strip washer. The washing is done four times with water using alternate dispensing and aspiration procedures. The plates are then dyed with 100 mL of 0.4% SRB for about 45 min. After dying the plates are again washed to remove the dye with 1% acetic acid and allowed to air dry overnight. 100 μL of 10 mM Tris base solution was added to each well of the plate and absorbance was measured at 570 nm using a 96 well plate reader (Tecan Spectra, Crailsheim, Germany). The IC_{50} value, defined as the concentrations of the compound at which 50% cell inhibition was observed, was estimated from the dose–response curves.

3. Results and discussion

3.1. Synthesis and characterization

The platinum(II) complexes **1**–**3** were synthesized in the reaction of $K_2[PtCl_4]$, R_2edda·2HCl and LiOH in water, molar ratio 1:1:2 (Scheme 1). The ligand precursors were synthesized using earlier described procedures [4,9].

Elemental analyses confirmed the molecular formulae. IR spectra of **1**–**3** show specific absorption bands characteristic for this family of compounds [4,5–8]. Thus, the band at 1734, 1741, 1742 cm^{-1} in the IR spectra of **1**–**3** were assigned to ester function

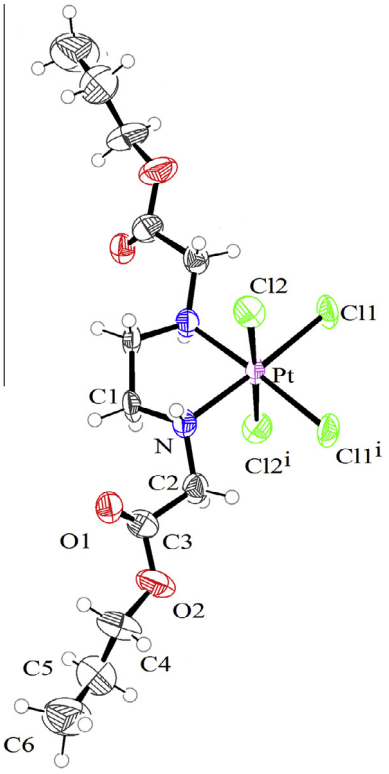


Fig. 3. ORTEP presentation of $[PtCl_4((n\text{-}Pr)_2edda)]$ (**4**).

Table 3
Selected bond lengths (Å) and angles (°) for **4**.

Bond lengths (Å)		Bond angles (°)	
Pt–Cl1	2.309(3)	Cl1–Pt–Cl2	91.2(1)
Pt–Cl2	2.317(2)	Cl1–Pt–N	177.0(2)
Pt–N	2.107(8)	N–Pt–N^i	84.5(3)
N–C1	1.49(1)	Cl1–Pt–$Cl1^i$	90.0(1)
N–C2	1.472(9)	Cl2–Pt–$Cl2^i$	178(2)
O2–C4	1.43(4)	Pt–N–C1	104.8(5)
O2–C3	1.34(2)	Pt–N–C2	115.4(5)
O1–C3	1.19(2)	C1–N–C2	113.0(6)
N–H3	0.98(2)	N–C1–$C1^i$	108.2(7)

Symmetry code: i = 1 − *x*,*y*,1/2 − *z*.

Table 4
Hydrogen-bond geometry (Å, °) for **4**.

D–H···A	D–H	H···A	D···A	D–H···A
N–H3···O1a^i	0.98(2)	2.48(2)	3.26(2)	137(1)
C1–H1···O1a	0.98	2.45	3.04(2)	118
C4a–H4a2···O1a	0.98	2.29	2.68(3)	103
C2–H4···Cl2	0.98	2.73	3.332(9)	120
C2–H5···Cl1	0.98	2.61	3.224(8)	120

Symmetry codes: a = 1 − *x*,*y*,1/2 − *z*; (1): a^i = 1 − *x*,1 − *y*,1 − *z*.

Table 2
Selected ^1H and ^{13}C NMR data (δ in ppm) for complexes **1**, **2** and **3**.

Complex	Isomer	^1H			^{13}C			
		CH_2-(en)	CH_2	CH_2O/CH_3O	CH_2-(en)	CH_2	CH_2O/CH_3O	COO
$[PtCl_2(Me_2edda)]$	**1a**	3.59 and 4.24	3.10	3.96	51.8	52.7	54.5	169.0
	1b	3.62 and 4.28		3.73				
$[PtCl_2(Et_2edda)]$	**2a**	3.71 and 4.18	2.79	4.18	53.4	55.0	61.6	169.1
	2b	3.92 and 4.33	3.16		54.1	55.9		169.2
$[PtCl_2(nPr_2edda)]$	**3a**	3.73 and 4.19	2.79	4.07	53.4	55.0	67.2	169.5
	3b	3.94 and 4.32	3.15		54.1	55.9		169.6

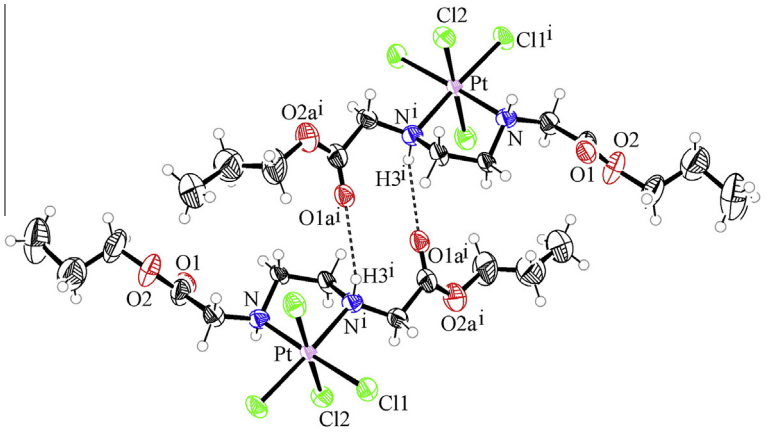


Fig. 4. ORTEP presentation of intermolecular hydrogen-bonding in [$PtCl_4$((*n*-Pr)$_2$edda)] (**4**).

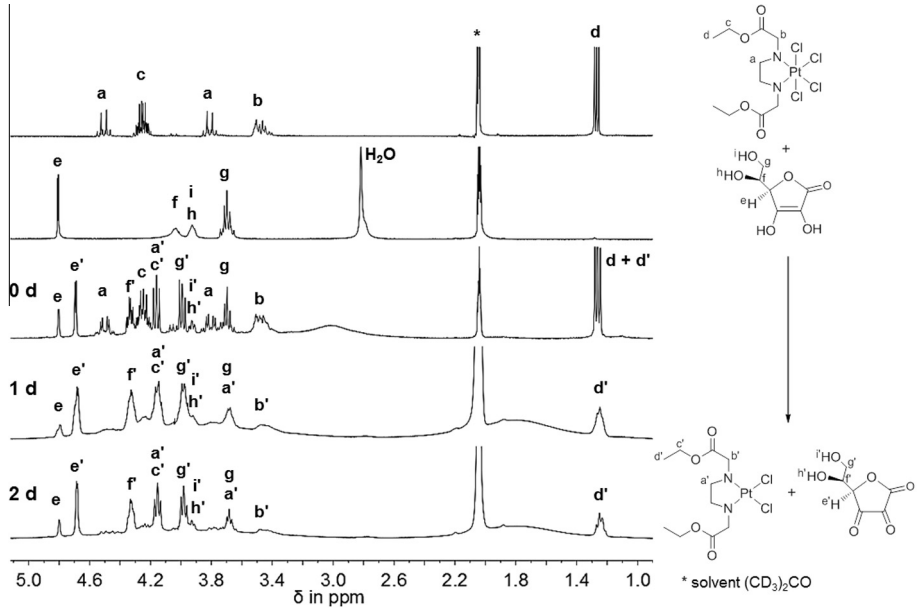


Fig. 5. Reduction of *rac*-[$PtCl_4$(Et_2edda)] with ascorbic acid followed by ^{1}H NMR spectroscopy.

ν(C=O), while those at 1220, 1218, 1224 cm^{-1} to ν(C–O) vibration, respectively. Methyl and methylene bands, ν(CH_2/CH_3), were found at 2984, 2969, 2976 cm^{-1} (for **1**–**3**). Indication of nitrogen coordination can be seen by the presence of a band for secondary amino groups (**1**–**3**: 3147, 3142, 3120 cm^{-1}) [4].

In ^{1}H and ^{13}C NMR spectra two sets of signals were found, as expected and previously described for analogous compounds in literature [6,7]. Thus, the complexes **1**–**3** exist in (*R*,*R*)/(*S*,*S*) and (*R*,*S*) diastereoisomeric. The enantiomeric (*R*,*R*)- and (*S*,*S*)-*N*,*N*′-configuration isomers, will give rise of one set of resonances. The diastereoisomer, (*R*,*S*)-*N*,*N*′ is expected to give rise of different chemical shifts at least for the hydrogen and carbon atoms near chiral *N* centers in comparison to (*R*,*R*)/(*S*,*S*)-*N*,*N*′ isomers. Previously, in some cases partial or total hydrolysis of the ester groups was observed and coordination occurred in κ*O*,κ^2*N*,*N*′ or κ^2*O*,*O*′,κ^2-*N*,*N*′ mode to metal ions [4,8]. Therefore, investigation regarding hydrolytic stability of ligands in water was performed (see 3.4).

As shown in Table 2, it is clear that most of the signals are double, and ester R groups mostly give a single set of resonances. The intensity ratio of signals vary, for complexes **1**, **2** and **3** ratio of the two isomers (*R*,*R*)/(*S*,*S*):(*R*,*S*) according to integrals are 2/3, 1/2 and 2/3, respectively. ^{1}H and ^{13}C NMR spectra are otherwise very similar to spectra of earlier characterized platinum complexes with R_2edda-type ligands [4,6,7]. Resonances of hydrogen atoms belonging to secondary amino groups are somewhat broad in the ^{1}H NMR spectra and appear at approximately 6.5 ppm for all complexes, while those of ethylene bridge hydrogen atoms show coordination induced shifts (0.1–0.9 ppm), which indicates that the coordination occurred *via* nitrogen atoms. In ^{13}C NMR spectra chemical shifts arising from ester carbon atoms of diastereoisomers are found at the expected positions for this class of compounds. Quantum chemical calculations of platinum(II) complexes have been performed and reported earlier [4]. Notably small differences in the total electronic energies between diastereoisomers (*R*,*R*)/(*S*,*S*) and (*R*,*S*) (ΔE_{tot} = −0.21 kcal/mol, ZPE corrected value) correlate with the coexistence of both diastereoisomers (see findings in ^{1}H NMR).

3.2. Crystal Structure of **4**

The platinum(IV) complex [$PtCl_4$((*n*-Pr)$_2$edda)], **4**, crystallized in monoclinic crystal system with space group *C*2/*c*. ORTEP presentation of the structure is given in Fig. 3, while bond lengths and angles are listed in Table 3. (*n*-Pr)$_2$edda ligand is coordinated to platinum(IV) ion through nitrogen atoms. Four chlorine atoms complete platinum(IV) inner sphere. X-ray structural analysis con-

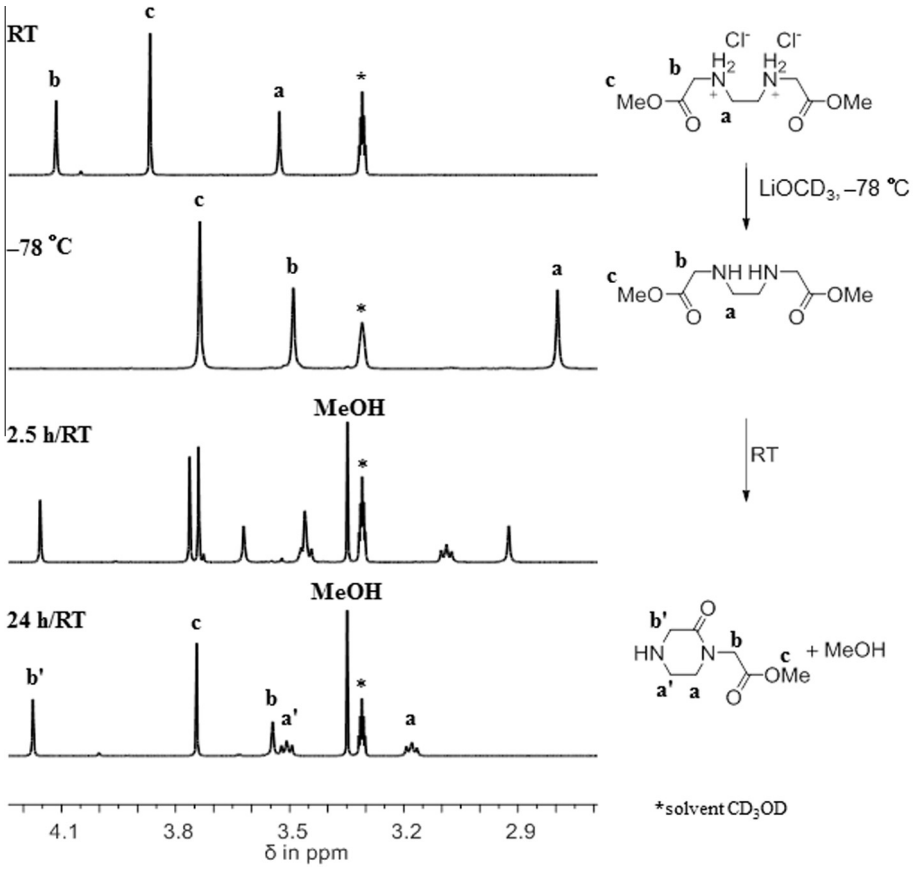


Fig. 6. ^{1}H NMR spectra of the cyclization reaction of Me_2edda.

Table 5
IC_{50} values[a] (μM) of platinum(II) complexes **1**–**3** in comparison with cisplatin.

	[$PtCl_2$(Me_2edda)], **1**	[$PtCl_2$(Et_2edda)], **2**	[$PtCl_2$(*n*-Pr)$_2$edda)], **3**	Cisplatin
518A2	91 ± 32	>125	52 ± 10	1.61 ± 0.21
8505C	104 ± 8	>125	58 ± 2	5.02 ± 0.23
A253	69 ± 14	68 ± 11	55 ± 1	0.81 ± 0.02
A431	62 ± 6	68 ± 11	55 ± 9	0.63 ± 0.03
A549	>125	>125	53 ± 3	1.51 ± 0.02
A2780	58 ± 7	56 ± 5	51 ± 1	0.55 ± 0.03
DLD-1	87 ± 26	>125	57 ± 1	5.14 ± 0.12
HCT-8	71 ± 15	>125	77 ± 10	1.53 ± 0.07
HT-29	58 ± 3	63 ± 7	69 ± 1	0.63 ± 0.03
MCF-7	69 ± 23	63 ± 7	55 ± 2	2.03 ± 0.11
SW1736	>125	>125	54 ± 6	3.20 ± 0.24

[a] Mean values ± SD (standard deviation) from three experiments; the compounds were incubated with cells for 96 h.

firmed that racemic mixture crystallized from mother liquor, namely the (*R*,*R*)/(*S*,*S*) enantiomeric pair was obtained.

The Pt–N and Pt–Cl distances are in the expected range for diaminetetrachloridoplatinum(IV) complexes [15]. Distances and angles of the ester moiety are comparable to those observed for ester precursors and analogous platinum(IV) complexes [4,5,7,16–19]. The Cl2–Pt–Cl2^i (see Fig. 3) angle is nearly linear (177.9(1)°). In the [NN^{i}PtCl1Cl1^i] plane, nitrogen atoms form a smaller angle with platinum (84.5(3)°) than in an ideal square, while chlorine atoms with platinum form an almost ideal (90.0(1)°) angle. The (ethylenediamine)Pt five-membered ring is twisted around C1–C1^i bond. The N and N^i atoms of the ligand each bear one hydrogen atom which are found in the electron density map.

Selected hydrogen bonds are listed in Table 4. These bonds stabilize the structure and lead to the packing manner as shown in Fig. 4. Intermolecular N–H3···O1 contacts are responsible for the layer formation, as they are the only intermolecular hydrogen bonds present. All other hydrogen bonds found in the structure of **4** (Table 4) are intramolecular.

3.3. Reduction of *rac*-[$PtCl_4$(Et_2edda)] with ascorbic acid

In order to provide correct assignation of diastereoisomers, the reduction of [$PtCl_4$(Et_2edda)] with ascorbic acid is performed and followed by time-depending ^{1}H NMR spectroscopy (Fig. 5). After two days platinum(IV) complex was completely reduced to the corresponding platinum(II) complex **2**, indicating a high possibility of the same outcome in living cells [8]. As earlier confirmed with X-ray structural analysis [$PtCl_4$(Et_2edda)] is obtained as racemic mixture [4], thus reduction should afford corresponding (*R*,*R*) and (*S*,*S*) isomers of platinum(II) complex **2**. Observation that only one set of signals is present in ^{1}H NMR spectra indicate that no isomerization

is occurring during reduction. Comparing chemical shifts in ^{1}H NMR spectra of synthesized complex **2** and reduction product of complex [$PtCl_4(Et_2edda)$] suggest that isomer **2a** represent (*R*,*R*)/(*S*,*S*)-*N*,*N*′ enantiomeric pair, while isomer **2b** (here not found) is the (*R*,*S*)-*N*,*N*′-configuration diastereoisomer.

3.4. Reactivity of ethylenediamine-N,N′-diacetate diesters

As mentioned in 3.1., in some cases in the synthesis of metal complexes with R_2edda-type ligands partial or total hydrolysis of the ester groups was observed [6,8]. Herein hydrolytic reactivity of R_2edda·2HCl is described. Whereas de-esterification of ligand precursor is very slow in D_2O, a fast side reaction occurred after complete neutralization of the hydrochlorides and further studies were performed. Dimethyl-ethylenediamine-*N*,*N*′-diacetate dihydrochloride monohydrate was suspended in deuterated methanol at −78 °C and deprotected with lithium methoxide. The free dimethyl ester was characterized by ^{1}H NMR spectroscopy. The NMR tube was stored at −78 °C for 45 min and a second spectrum showed only small changes, clearly indicating deprotonation of nitrogen. A fast intramolecular reaction occurred at room temperature in quantitative yield (Fig. 6). The product was characterized after 2.5 and 24 h by ^{1}H NMR spectroscopy [9,20]. Complete neutralization of the dihydrochlorides enabled a fast intramolecular reaction, in the case of Me_2edda which was confirmed by ^{1}H NMR spectroscopy (Fig. 6). These findings explain why the yield of some of our reactions was not always a κ^2NN' coordination to metal ion [4,8]. R_2edda-type ligands are stable as dihydrochlorides in solid form over long period [4–7]. In solution they hydrolyze slowly with half-life of 2 months. Upon neutralization intramolecular amide formation occurs very fast pointing high reactivity of nonprotonated R_2edda-type esters.

3.5. In vitro antitumoral studies

The *in vitro* cytotoxic activity of **1**–**3** was studied on 11 tumor cell lines, 5182A (melanoma), 8505C (human thyroid carcinoma), A253 (head and neck tumor), A431 (cervix), A549 (lung), A2780 (ovarian), MCF-7 (breast) and HT-29, HCT-8, DLD-1, SW1736 (all colon) by the SRB colorimetric assay method. The IC_{50} values of each compound on these cell lines are given in Table 5. Higher cytotoxic potential can be observed when methyl or ethyl group is substituted with *n*-propyl ester group (**1**, **2** → **3**), with exception of HCT-8 and HT-29 cells. Complex **3** showed the highest action against ovarian (A2780) cells with an IC_{50} value of 51 ± 1 μM.

Platinum(IV) complexes with same bidentate ligands were also studied for *in vitro* cytotoxic action [4], and it was found that the activity of the complexes depends upon the number of carbon atoms of the ester chain, from which it could be inferred that the longer the chain the greater the activity. When comparing antitumor action between platinum(II) and platinum(IV) complexes it can be seen clearly that in case of A549 and 518A2 cells [4] Me_2edda and Et_2edda platinum(IV) complexes are more active than corresponding platinum(II) complexes, but as in the case of platinum(IV) complexes also platinum(II) complex containing $(n\text{-Pr})_2$edda ligand is the most active complex on the selected cell line. On the other hand, DLD-1 cells are more resistant to platinum(IV) complexes. However, compared to cisplatin (Table 5) all tested compounds show moderate or low cytotoxic activity.

4. Conclusions

Synthesis of three novel complexes of platinum(II) with R_2edda (R = Me, Et, *n*-Pr; edda = ethylenediaminediacetate), [$PtCl_2(R_2$edda)] is described and they are characterized by IR and NMR spectroscopy and elemental analysis. All complexes were found in stereoisomeric forms ((*R*,*R*), (*S*,*S*), and (*R*,*S*)≡(*S*,*R*)) as confirmed by NMR spectroscopy which is in agreement with DFT calculations [4]. Crystal structure of a platinum(IV) complex [$PtCl_4((n\text{-Pr})_2$edda)], **4**, is solved and given. The coordination occurred *via* nitrogen atoms, and bond lengths and angles are comparable to those of same compounds of this class. Reaction of the racemic mixture (*R*,*R*)/(*S*,*S*)-[$PtCl_4(Et_2$edda)] with ascorbic acid is observed by ^{1}H NMR spectroscopy. Formed product (*R*,*R*)/(*S*,*S*)-[$PtCl_2(Et_2$edda)] allowed the correct assignation of diastereoisomers. Studies of dialkyl-ethylenediamine-*N*,*N*′-diacetate pointed to hydrolysis and cyclization reactions in solution. The *in vitro* cytotoxic activity of complexes, **1**–**3**, was studied on 11 tumor cell lines, 5182A (melanoma), 8505C (human thyroid carcinoma), A253 (head and neck tumor), A431 (cervix), A549 (lung), A2780 (ovarian), MCF-7 (breast) and HT-29, HCT-8, DLD-1, SW1736 (colon). Tested compounds showed lower cytotoxicity than corresponding platinum(IV) complexes and cisplatin.

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Appendix A. Supplementary data

CCDC 976233 contains the supplementary crystallographic data for **4**. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336 033; or e-mail: deposit@ccdc.cam.ac.uk.

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